CHAPTER 6

Size of sentinel node metastasis and chances of non-sentinel node involvement and survival in early-stage vulvar cancer: results from GROINSS-V, a multicentre observational study

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Summary

Background

Currently, all patients with vulvar cancer with a positive sentinel node undergo inguinofemoral lymphadenectomy, irrespective of the size of sentinel node metastases. Our study aimed to assess the association between size of sentinel node metastasis and risk of metastases in non-sentinel nodes, and risk of disease-specific survival in early-stage vulvar cancer.

Methods

In the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V), sentinel node detection was done in patients with T1 / T2 (< 4cm) squamous cell vulvar cancer, followed by inguinofemoral lymphadenectomy if metastatic disease was identified in the sentinel node, either by routine examination or pathological ultrastaging. For the present study, sentinel nodes were independently reviewed by two pathologists.

Findings

Metastatic disease was identified in one or more sentinel nodes in 135 (33%) of 403 patients, and 115 (85%) of these patients had Inguinofemoral lymphadenectomy. The risk of non-sentinel node metastases was higher when the sentinel node was found to be positive with routine pathology than with ultrastaging (23 of 85 groins (27%) *vs* three of 56 groins (5%), p=0.001). For this study, 723 sentinel nodes in 260 patients (2.8 sentinel nodes per patient) were reviewed. The proportion of patients with non-sentinel node metastases increased with size of sentinel node metastasis; one of 24 patients with individual tumour cells had a non-sentinel node metastasis (4.2%); two of 19 (10.5%) with metastases ≤ 2mm; two of 15 (13.3%) with metastases > 2 – 5mm; and ten of 21 (47.6%) with metastases > 5mm. Disease-specific survival for patients with sentinel node metastases > 2mm was lower than for those with sentinel node metastases ≤ 2mm (69.5% *vs* 94.4%, p=0.001).

Interpretation

Our data show that the risk of non-sentinel node metastases increases with size of sentinel node metastasis. No size cut-off seems to exist below which chances of non-sentinel node metastases are close to zero. Therefore, all patients with sentinel node metastases should have additional groin treatment. The prognosis for patients with sentinel node metastasis larger than 2mm is poor, and novel treatment regimens should be explored for these patients.

Introduction

Introduction of the sentinel node procedure is one of the most promising recent advances in surgical management of early-stage squamous cell cancer of the vulva: we have previously shown that inguinofemoral lymphadenectomy can be avoided when the sentinel node is negative for disease, resulting in a significant decrease in morbidity.[1]

Pathological ultrastaging (multiple sectioning and immunohistochemistry) is typically done on excised sentinel nodes, and substantially improves detection of sentinel node metastases.[2,3] For most patients with vulvar cancer with a positive sentinel node, metastases in the non-sentinel nodes are absent.[4,5] In patients with breast cancer, micrometastases are defined by size, with the maximum dimension of the largest lymph node tumour no larger than 2.0mm. Submicrometastases are no larger than 0.2mm.[6] Patients with breast cancer with micrometastases in the sentinel node have a significantly lower risk of non-sentinel node involvement compared with patients with macrometastases.[7-9] In the tumour-node-metastasis (TNM) classification for breast cancer, submicrometastases are classified as N0 and patients are treated as lymph node-negative. Patients with cutaneous melanoma with micrometastases smaller than 0.1mm also have a lower risk of non-sentinel node involvement than those with larger sentinel-node metastases and the same prognosis as sentinel node-negative patients.[10] In vulvar cancer, comparable data are not available and, until now, no distinction has been made between micrometastases and macrometastases with regard to additional treatment.

The presence or absence of inguinofemoral lymph node metastases is the most important prognostic factor in vulvar cancer;[11-13] however, the absence of prospective data has led some to question the clinical significance of micrometastases in sentinel nodes. In the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V), all early-stage vulvar cancer patients with a positive sentinel node (found by routine pathology or pathological ultrastaging) were routinely scheduled for inguinofemoral lymphadenectomy. Pathological ultrastaging was included in the original study protocol and was done when the sentinel nodes was found to be negative on routine haematoxylin and eosin examination.

The aim of the present study was to assess the association between size of sentinel node metastasis and risk of metastases in non-sentinel nodes, as well as disease-specific survival, in patients with early-stage vulvar cancer. We compared the risk of non-sentinel node involvement and disease-specific survival in patients with a positive sentinel node found by routine pathology versus ultrastaging, since metastases identified by routine examination are larger than those identified by ultrastaging. This analysis was done for all GROINSS-V patients (n=403). We also did a pathology review, which allowed a more detailed analysis of absolute size of sentinel node metastases in relation to the risk of non-sentinel node involvement and disease-specific survival.

Methods

Patients

A prospective multicenter observational study (GROINSS-V) was done in patients with early-stage squamous cell vulvar cancer (diameter < 4cm) between 2000 and 2006. Central data management was done at the University Medical Centre Groningen, the Netherlands. Treatment consisted of excision of the primary tumour and the sentinel node procedure (using radioactive tracer and blue dye). In case of a positive sentinel node, the protocol specified inguinofemoral lymphadenectomy by separate incisions. Follow-up schedules for patients with positive sentinel nodes were at the discretion of the participating institute, but generally consisted of gynaecological examination and palpation of the groins at gradually increasing intervals. The outcome for patients with negative sentinel nodes was reported previously.[1] For analysis of the prognostic value of metastatic sentinel nodes, follow-up data for all patients from GROINSS-V who had metastatic sentinel nodes were updated until January, 2009. Permission for the study was obtained from all local ethics committees, and all patients gave written informed consent.

Procedures

The sentinel node procedure was done as previously described.[1] In case of a positive sentinel node, unilateral or bilateral inguinofemoral lymphadenectomy was done by separate incisions; either at the same procedure, when frozen sectioning (optional) showed sentinel node metastases, or in a second procedure, when definitive histological examination showed metastatic disease. The node-bearing fat pad was removed by separate incisions parallel to the inguinal ligament. The anatomical margins of dissection were the inguinal ligament cephalad, the adductor longus muscle medially, and the sartorius muscle inferolaterally. After opening of the cribiform fascia, all node-bearing fatty tissue medial of the femoral vein was also removed. Standard local treatment consisted of radical local excision of the vulvar tumour with macroscopically tumour-free margins of at least 1cm. Indications for adjuvant radiotherapy to the groins were more than one metastatic lymph node, extracapsular tumour growth, or both. The radiotherapy protocol was at the discretion of the participating institution.

The histological work-up of sentinel nodes was the same for all participating centres and was done as previously described.[1] Intraoperative frozen sectioning was performed by cutting the sentinel node in half along the long axis of the node. One half was snap frozen in isopentane and a superficial standard frozen section was cut. All lymph nodes from the lymphadenectomy specimens were studied individually. Sections were made at 500µm intervals for haematoxylin and eosin staining. No ultrastaging was done for the non-sentinel nodes. Measurement of the size of sentinel node metastasis was not part of the original GROINSS-V study protocol. To study the association between size of sentinel node metastasis and risk of non-sentinel node metastases for this study,

pathological review of sentinel nodes was done by two specialist gynaecological pathologists. These reviewers were masked to clinical data and the results of original histological assessment. All slides of the sentinel nodes were reviewed (frozen sections, routine haematoxylin and eosin slides, multiple sections, and immunohistochemistry slides). Metastases were defined as clusters of tumour cells of any size detected on haematoxylin and eosin slides or at immunohistochemistry, or isolated tumour cells detected at immunohistochemistry. Anucleate keratin-positive structures were not considered positive. The diameter of the sentinel node metastasis and presence of extranodal growth were recorded. For logistical reasons, this analysis was limited to the Dutch participating centres (n=307).

Statistical Analysis

Per-groin analysis was done to assess the risk of non-sentinel node involvement. The Kaplan-Meier method was used for survival analyses. The log-rank test was applied to compare survival between different categories of patients. Cox proportional-hazards model was applied to estimate the effect of prognostic factors on disease-specific survival. Follow-up and survival were calculated from the date of primary surgery to the data of last examination or death. P values < 0.05 were considered significant. Statistical analyses were done with SPSS software version14.

Role of the Funding Source

There was no funding source for this study. The corresponding author had full access to all data and had final responsibility to submit for publication.

Results

From March 6, 2000, until June 7, 2006, 403 eligible patients underwent the sentinel node procedure according to the GROINSS-V protocol. In 135 (33%) of 403 patients (164 groins) original pathological assessment showed metastatic disease in one or more sentinel nodes. The baseline characteristics of these 135 patients are summarised in Table 1. Frozen sectioning was done in 315 (78%) of 403 patients and showed a sensitivity of 48% (95% CI, 38 – 57), specificity of 100% (95% CI, 98 – 100), negative predictive value of 78%, and a positive predictive value of 100%. Routine histological examination detected a positive sentinel node in 80 (59%) of 135 patients (98 [60%] of 164 groins). Ultrastaging detected a positive sentinel node in 55 (41%) of 135 patients (66 [40%] of 164 groins). After multiple sectioning, immunohistochemistry identified micrometastases in 36 (12%) of 304 patients with a negative sentinel node. Inguinofemoral lymphadenectomy was done in 115 (85%) of 135 patients with positive sentinel nodes (141 sentinel node positive groins; 85 positive by routine pathology, 56 positive by ultrastaging).

Table 1. Patient and Primary Tumour Characteristics of All Sentinel Node Positive Patients (n=135)

Characteristics	Number of patients (%)
Age	
≤ 70	59 (43%)
> 70	76 (56%)
Diameter primary tumour	
≤ 2.0cm (T1)	46 (34%)
> 2.0cm (T2)	87 (64%)
Unknown	2 (1%)
Depth of invasion	
≤ 5.0mm	62 (46%)
> 5.0mm	63 (47%)
Unknown	10 (7%)
Location of the primary tumour	
Lateralized	42 (31%)
Midline	93 (69%)
Multifocal/Unifocal	
Multifocal	11 (8%)
Unifocal	124 (92%)
Treatment primary tumour	
Radical vulvectomy	15 (11%)
Wide local excision	118 (87%)
Radiotherapy	2 (1%)
Uni- or bilaterally positive SN	
Unilateral	106 (79%)
Bilateral	29 (21%)
SN found positive by	
Routine histological examination	80 (59%)
Ultrastaging	55 (41%)
Additional treatment	
Inguinofemoral lymphadenectomy	115 (85%)
Unilateral	*33(29%)*
Bilateral	*82 (71%)*
Radiotherapy	14 (10%)
No additional treatment	6 (4%)
Non-sentinel node metastases	
No non-sentinel node metastases	91 (67%)
Non-sentinel node metastases present	24 (18%)
Unknown*	20 (15%)

SN = sentinel node. *Unknown because no complete lymphadenectomy was performed.

Fourteen patients received radiotherapy to the groins instead of lymphadenectomy, for various reasons (eg, advanced age, or co-morbidity). Six patients had no additional treatment: three patients refused additional treatment, two patients died of a non-cancer related cause before additional treatment could be started, and one had osteomyelitis of the symphysis, resulting in substantial delay of additional treatment.

Non-sentinel node metastases were found in 24 (21%) of 115 patients and 26 (18%) of 141 groins. The risk of non-sentinel node metastases was higher when the sentinel node was found positive by routine histological assessment (23 of 85, 27%) than by ultrastaging (3 of 56, 5%; p=0.001, calculated per groin; Figure 1A).

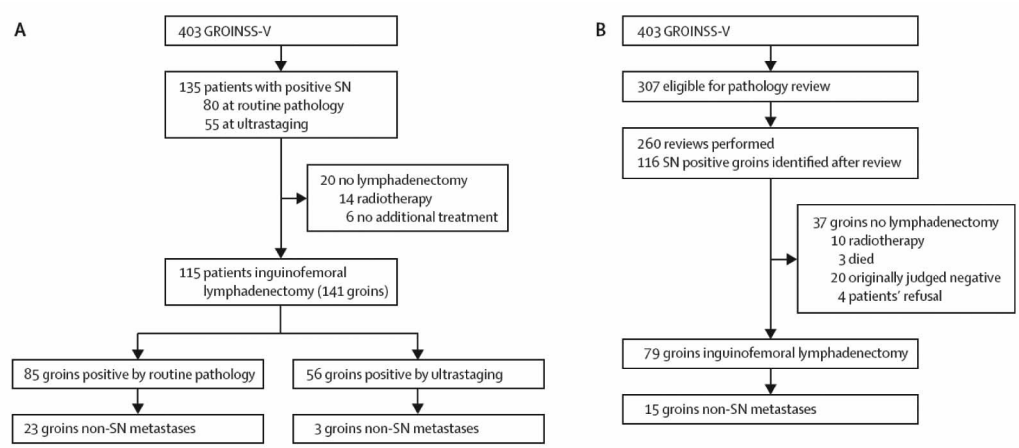

Figure 1. Flow-Chart for Analysis of all Patients from GROINSS-V with a Positive Sentinel Node (A) and of Patients with Pathology Review (B)

After a median follow-up of 31 months (range 0 – 109), persistent or recurrent disease was noted in 55 (40.7%) of 135 patients: 34 patients had local recurrence, 11 had groin recurrence, seven had distant recurrence, and three had persistent disease despite treatment. Median time to recurrence was 16 months (range 2 – 67) for patients with local recurrences, 9 months (range 2 – 14) for patients with groin recurrence, and 8 months (range 4 – 26) for patients with distant recurrence. During follow-up, 15 of 135 patients (11%) died of intercurrent disease, while 28 of 135 patients (21%) died of disease. Three of 28 patients were never free of disease and died 3, 3, and 6 months after start of treatment; all had widespread metastatic disease in non-sentinel nodes and locoregional progression

despite surgery and radiotherapy. Local recurrences were observed in 34 (25%) of 135 patients and treated by radical local excision with inguinofemoral lymphadenectomy, radiotherapy, or both. Ten of 34 (30%) patients with local recurrence died of disease, at a median time after primary treatment of 15 months (range 7 – 43).

Eleven patients were diagnosed with a solitary groin recurrence. Seven patients were diagnosed with recurrence after inguinofemoral lymphadenectomy, and two after radiotherapy alone for a positive sentinel node. One patient developed groin recurrence after adjuvant treatment for a metastatic sentinel node was delayed because of osteomyelitis, and one patient developed groin recurrence in a sentinel node negative groin (lymphadenectomy performed in contralateral groin because of a metastatic sentinel node). Groin recurrence was treated with a combination of surgery and radiotherapy, or radiotherapy alone. Nine of 11 patients with groin recurrence died of disease, at a median time after primary treatment of 12 months (range 5 – 24). Seven patients were diagnosed with isolated distant recurrences (three with pelvic recurrences) and were managed with varying palliative radiotherapy and chemotherapy regimens. Six patients with distant recurrences died of disease (6, 7, 8, 8,13, and 14 months after primary treatment) and one patient is alive with disease 26 months after primary treatment (five months after diagnosis of pelvic recurrence).

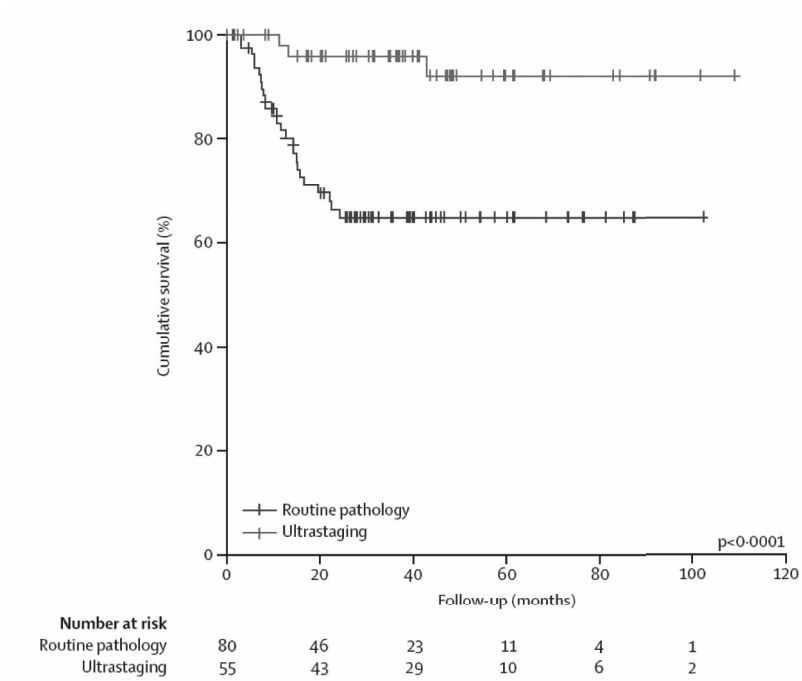

Figure 2. Disease-Specific Survival of Patients with Vulvar Cancer and a Positive Sentinel Node, as Found by Routine Pathology versus Ultrastaging

Five-year disease-specific survival for patients with positive sentinel nodes was 77.3%; 64.9% when identified by routine pathology versus 92.1% when identified by ultrastaging ($p < 0.0001$; Figure 2). Groin and distant recurrence was more common when the sentinel node was found positive by routine pathology ($p=0.005$). No association was found between the size of sentinel node metastases and local recurrence. Tumour diameter, depth of invasion, and number of metastatic lymph nodes were also not associated with recurrent disease (Table 2).

Table 2. Recurrence According to Characteristics in Sentinel Node Positive Patients (n = 132*)

Variable	Recurrence rate (%)	Local recurrence rate (%)	Groin/distant recurrence rate (%)
Size primary tumour			
T1	16 / 46 (34%)	12 / 46 (26%)	4 / 46 (9%)
T2	35 / 84 (42%)	22 / 84 (26%)	13 / 84 (15%)
P value†	0.44	0.99	0.27
Depth of invasion			
≤ 5	20 / 62 (32%)	12 / 62 (19%)	8 / 62 (13%)
> 5	27 / 60 (45%)	18 / 60 (30%)	9 / 60 (15%)
P value†	0.15	0.17	0.74
No. of positive nodes			
≤ 1 lymph nodes	24 / 69 (35%)	16 / 69 (23%)	8 / 69 (12%)
> 1 lymph nodes	28 / 63 (44%)	18 / 63 (29%)	10 / 63 (16%)
P value†	0.26	0.48	0.47
SN positive by			
Routine	37 / 77 (48%)	21 / 77 (27%)	16 / 77 (21%)
Ultrastaging	15 / 55 (27%)	13 / 55 (24%)	2 / 55 (4%)
P value†	0.016	0.64	0.005

SN = sentinel node. * Three patients with persistent disease after primary treatment were excluded.
†Chi-square test.

Pathology review of the sentinel nodes was performed for 260 (85%) of 307 patients from Dutch centres. The slides of 47 patients (from two centres) could not be completely retrieved and these patients were excluded from analysis. 723 sentinel nodes were reviewed (mean of 2.8 nodes per patient). Pathological review was discordant with the initial assessment in 48 (7%) of 723 sentinel nodes; 32 sentinel nodes were scored as false-negative and 16 as false-positive. In all false-negative sentinel nodes, only isolated tumour cells were missed (1 – 100 cells). In all false-positive sentinel nodes, anucleate keratin-positive structures were observed. Sentinel node metastases initially detected by ultrastaging were significantly smaller than metastases initially detected by routine pathology (Table 3).

Analyses per groin were done to assess the risk of additional metastases. Pathology review identified 116 groins with positive sentinel nodes. Inguinofemoral lymphadenectomy was done in 79 (68%) of 116 positive groins. Reasons for no lymphadenectomy were: radiotherapy instead of inguinofemoral lymphadenectomy (n=10), death before start of additional treatment (n=3), only sentinel nodes positive at review (n=20), and patient refused additional therapy (n=4; Figure 1B). Non-sentinel node metastases were found in 15 of 79 groins (19%; Table 4). Chances of additional groin metastases were the same for patients with unilateral sentinel node metastases compared with bilateral sentinel node metastases (10 of 51 groins [19.6%] *vs* five of 28 groins [17.9%]). The presence of non-sentinel node involvement was not related to the number of positive sentinel nodes per groin (p = 0.11). The risk of additional non-sentinel node metastases increased with size of the sentinel node metastasis. No cut-off size was found below which chances of non-sentinel node metastases were close to zero, although the risk of non-sentinel node metastases associated with isolated tumour cells in the sentinel node seems to be low.

Table 3. Initial Method of Detection and Actual Size of Sentinel Node Metastasis*

	Routine Pathology (number of groins)	Ultrastaging (number of groins)	Total
ITC	0	28	28
≤ 1mm – 2mm	12	13	25
> 2mm – 5mm	14	1	15
> 5mm	25	0	25
Total	51	42	93

Size of all tumours detected by ultrastaging versus routine pathology: P < 0.0001.
ITC = isolated tumour cells. *Analyzed for groins in which initial pathology was positive and review of sentinel nodes was done (93 groins).

Survival was strongly associated with size of sentinel node metastases: 5-year disease-specific survival was 97% for patients with isolated tumour cells in the sentinel node; 88% for those with sentinel node metastases ≤ 2mm; 70% for those with metastases > 2 – 5mm; and 69% for those with metastases > 5mm (p=0.012; Figure 3). Disease-specific survival for patients with sentinel node metastases larger than 2mm was lower than for those with metastases ≤ 2mm (69.5% *vs* 94.4%, p=0.001). A Cox proportional-hazards model showed that disease-specific survival was related to size of sentinel node metastases, independent of the number of positive nodes (HR 6.4, 95% CI 1.7 – 23.6; p=0.006). None of the patients with a false-negative sentinel node had groin recurrence (n = 20 groins, no lymphadenectomy).

Table 4. Risk of Non-Sentinel Node Metastases by Largest Tumour Burden in the Sentinel Node

	Number of SN-positive groins	Number of SN-positive groins with IFLA	Number of groins with non-SN metastases	Non-SN metastases (% per groin)*
ITC	51	24	1	4.2%
≤ 1 mm	13	10	1	10%
> 1 – 2 mm	12	9	1	11.1%
> 2 – 5 mm	15	15	2	13.3%
> 5 – 10 mm	16	13	5	38.5%
> 10 mm	9	8	5	62.5%
Total	116	79	15	19.0%

SN = sentinel node. IFLA = inguinofemoral lymphadenectomy. ITC = isolated tumour cells. * Analyzed for groins in which IFLA was done.

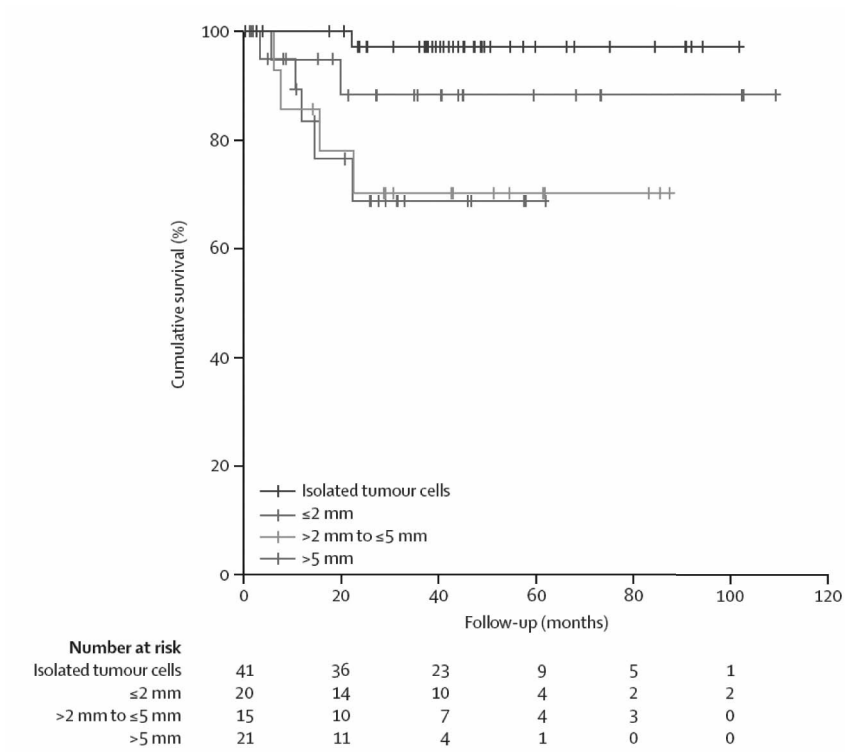

Figure 3. Disease-Specific Survival of Patients with a Positive Sentinel Node, by Size of Metastases

Discussion

The results of this study suggest that identification of sentinel node metastasis in early-stage vulvar cancer necessitates further groin treatment, regardless of the size of the metastasis. We did not find a cut-off size for sentinel node metastasis below which the risk of additional groin metastases becomes negligible.

To our knowledge, this is the first study to investigate the association between risk of non-sentinel node metastases and size of sentinel node metastases in vulvar cancer. In breast cancer, size of sentinel node metastases has already been incorporated into staging classification. De Boer and colleagues[14] recently reported that breast cancer patients with isolated tumour cells in the sentinel node had lower disease-free survival than those with a negative sentinel node. However, other large prospective studies of patients with breast cancer have shown no effect on disease-free survival with the identification of isolated tumour cells,[15] or with both isolated tumour cells and micrometastases.[16] An important issue when comparing and extrapolating results from breast to vulvar cancer is that in breast cancer the decision for adjuvant systemic therapy is based on primary tumour and patient characteristics, rather than nodal status alone. Such adjuvant therapy might eradicate non- sentinel node metastases in patients who do not undergo axillary lymph node dissection, resulting in excellent locoregional control. This hypothesis is supported by very low axillary recurrence after identification of a negative sentinel node (0.13 – 1.2%),[17] despite an accepted false-negative rate for sentinel node dissection in breast cancer of 7-8%. Comparable, effective systemic adjuvant treatment in vulvar cancer is currently not available, and control of locoregional lymph nodes requires either surgery or radiotherapy. Additionally, unlike in breast cancer where nodal recurrences are amenable to various therapeutic options, groin recurrences in vulvar cancer are often fatal.[18] Our present study shows the presence of additional metastases in non-sentinel nodes in 4.2% (95% CI, 0.1 – 21.1%) of patients with isolated tumour cells in the sentinel node. Based on a previously published groin recurrence rate of 2.9% in patients with a negative sentinel node, and data from the present study for patients with a positive sentinel node, we recommend additional groin treatment (with surgery as first choice) for all patients with vulvar cancer with sentinel node metastasis, regardless of size. A second observational study, GROINSS-V-II, is investigating radiotherapy to the groins instead of inguinofemoral lymphadenectomy as additional treatment in patients with vulvar cancer and positive sentinel nodes.

Our study shows that the size of sentinel node metastases is a significant predictor of disease-specific survival in early vulvar cancer. Origoni and co-workers[19] showed that the size of lymph node metastases significantly correlated with survival in patients with advanced (stage III – IVA) vulvar cancer. For patients with one positive lymph node, the most important prognostic factor was the greatest dimension of metastasis within the lymph node.[20] No comparable studies have been done for sentinel nodes in early vulvar cancer. The poor prognosis of patients with sentinel node

metastases > 2mm supports the case for more aggressive treatment in this group, eg, by combining radiation with chemotherapy. Because this approach might lead to more treatment-associated morbidity, it should preferably be limited to high-risk patients.

In our study, patients with isolated tumour cells in the sentinel node had a similar prognosis to patients with a negative sentinel node, as reported previously.[1] In small retrospective studies of patients with vulvar cancer who were managed with inguinofemoral lymphadenectomy, additional immunohistochemical assessment of lymph nodes originally judged to be negative by standard haematoxylin and eosin staining resulted in upstaging in 23 – 42% of patients, whereas no differences in survival were noted between patients with and without such micrometastases.[21,22] Despite these observations and those from our own study, we cannot conclude that inguinofemoral lymphadenectomy can be omitted in patients with only isolated tumour cells, since the therapeutic effect of this procedure, which was part of routine treatment in our study, is undefined.

Although our study is the largest currently available on sentinel nodes in vulvar cancer, there are some limitations. First, a disparity of 7% was found between original pathological assessment and review. Roberts and colleagues[23] showed that the ability of pathologists to recognise metastases decreases with decreasing number of tumour cells in a sentinel node, which could bias the results in a multicenter study without central pathological review. Second, despite the large number of patients in this study, the numbers of metastases in each size category detected by routine pathology or ultrastaging (Table 3) were small. Finally, for each groin, we only assessed the largest metastatic focus in a sentinel node. Other aspects of sentinel node pathology might also affect the chances of non-sentinel node metastases (eg, total square area of metastatic disease and number of metastatic foci in the sentinel node). The effect of bilateral involvement was not taken into account, but compared with patients with one positive sentinel node, we did not find more non-sentinel node metastases in patients with a positive sentinel node in both groins.

In conclusion, non-sentinel node metastases occur more often as the size of sentinel node metastasis increases. Our analysis did not show a clear cut-off with respect to tumour load in the sentinel node, below which no non-sentinel node metastases were observed. Therefore, all patients with sentinel node metastases require additional groin treatment. Tumour load in the sentinel node was strongly associated with survival. Since the prognosis for patients with sentinel node metastasis larger than 2mm is poor, novel treatment regimens, such as chemoradiation, should be explored.

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